

In re Application of DENSLOW et al.  
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### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### Listing of Claims:

1. (Currently Amended) A method for detecting the presence of an agent having estrogenic or androgenic activity in a sample, the method comprising the steps of:
  - (A) providing at least one fish cell which was exposed to the sample;
  - (B) analyzing the at least one fish cell for expression of at least one gene wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558; and
  - (C) comparing the expression of the at least one gene in the cell compared to the expression of the at least gene in a control cell not exposed to the sample or an agent having estrogenic or androgenic activity, wherein a difference in the expression of the at least one gene in the at least one fish cell compared to the expression of the at least one gene in the control cell indicates that the sample contains an agent having estrogenic or androgenic activity.
2. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least two different genes each being wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.
3. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least three different genes each being wholly or partially encoded by a nucleotide sequence

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selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

4. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least four different genes each being wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

5. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least ten different genes each being wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

6. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least twenty-five different genes each being wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

7. (Currently Amended) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises analyzing the cell for expression of at least one hundred different genes each being wholly or partially encoded by a nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25,

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28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

8. (Original) The method of claim 1, wherein the at least one fish cell is a large mouth bass cell.

9. (Original) The method of claim 1, wherein the at least one fish cell is a sheep's head minnow cell.

10. (Original) The method of claim 1, wherein the at least one fish cell was obtained from a fish that had been exposed to the sample.

11. (Original) The method of claim 1, wherein the step of analyzing the at least one fish cell for expression of at least one gene comprises isolating RNA transcripts from the at least one cell.

12. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least one probe that hybridizes under stringent hybridization conditions to at least one nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

13. (Original) The method of claim 12, wherein the at least one probe is immobilized on a substrate.

14. (Original) The method of claim 13, wherein the substrate is comprised of a substance selected from the group consisting of: nylon, nitrocellulose, glass, and plastic.

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15. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least two different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NO's: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

16. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least three different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NO's: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

17. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least four different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NO's: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

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18. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least ten different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

19. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least twenty-five different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

20. (Currently Amended) The method of claim 11, wherein the step of analyzing the at least one fish cell for expression of at least one gene further comprises contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least one-hundred different probes that each hybridize under stringent hybridization conditions to a different nucleotide sequence selected from the group consisting of ~~SEQ ID NOs: 1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

21. (Original) The method of claim 12, wherein the at least one probe is conjugated with a detectable label.

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22. (Original) The method of claim 21, wherein the isolated RNA transcripts or nucleic acids derived therefrom are conjugated with a detectable label.

23. (Currently Amended) The method of claim 1, further comprising analyzing the control cell not exposed to the sample or an agent having estrogenic or androgenic activity for expression of at least one gene wholly or partially encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

24. (Currently Amended) The method of claim 23, wherein the step of analyzing the control cell for expression of at least one gene further comprises isolating RNA transcripts from the control cell and contacting the isolated RNA transcripts or nucleic acids derived therefrom using the isolated RNA transcripts as templates with at least one probe that hybridizes under stringent hybridization conditions to at least one nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558.

25. (Original) The method of claim 24, wherein the RNA transcripts or nucleic acids derived therefrom isolated from the at least one fish cell are conjugated with a first detectable label and the RNA transcripts or nucleic acids derived therefrom isolated from the control cell are conjugated with a second detectable label differing from the first detectable label.

26. (Currently Amended) The method of claim 23, further comprising isolating RNA transcripts from the at least one fish cell and contacting the RNA transcripts isolated from the at least one fish cell or nucleic acids derived therefrom using the RNA transcripts isolated from the at least one fish cell as templates with at least one molecule that hybridizes under stringent

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conditions to at least one nucleotide sequence selected from the group consisting of ~~SEQ ID~~  
NOs: ~~1-560~~ SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167,  
178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529,  
555, and 558.

27. (Original) The method of claim 26, wherein the at least one probe is conjugated with a first detectable label and the at least one molecule is conjugated with a second detectable label differing in chemical structure from the first detectable label.

28. (Original) The method of claim 27, wherein the step of comparing the expression of the at least one nucleic acid in the cell compared to the expression of the at least one nucleic acid in a control cell not exposed to the sample or an agent having estrogenic or androgenic activity comprises quantifying the amount of first detectable label associated with the RNA transcripts isolated from the control cell or nucleic acids derived therefrom, and quantifying the amount of second detectable label associated with the RNA transcripts isolated from the at least one fish cell or nucleic acids derived therefrom.

29. (Original) The method of claim 1, further comprising the step of contacting the at least one fish cell with the sample prior to the step of analyzing the at least one fish cell for expression of the at least one gene.

30. (Original) The method of claim 1, wherein the sample comprises water.

31. (Original) The method of claim 1, further comprising the steps of:  
providing a fish;  
contacting the fish with the sample; and  
isolating the at least one fish cell from the fish contacted with the sample.

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32. (Currently Amended) A method for determining whether an agent has estrogenic, anti-estrogenic, androgenic or anti-androgenic activity, the method comprising the steps of:

providing at least one fish cell;

contacting the at least one fish cell with the agent;

analyzing the at least one fish cell for expression of at least one gene wholly or partially encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 SEQ ID NO's: 14, 15, 25, 28, 30, 42, 44, 47, 52, 61, 62, 71, 146, 148, 149, 166, 167, 178, 194, 199, 200, 207, 285, 347, 424, 489, 505, 509, 516, 519, 532-534, 542, 545, 551, 529, 555, and 558; and

comparing the expression of the at least one gene in the cell compared to the expression of the at least one nucleic acid in a control cell not exposed to the sample or an agent having estrogenic or androgenic activity, wherein a difference in the expression of the at least one nucleic acid in the at least one fish cell compared to the expression of the at least one nucleic acid in the control cell indicates that the agent has estrogenic, anti-estrogenic, androgenic, or anti-androgenic activity.

33. (Withdrawn) A substrate having immobilized thereon at least one nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

34. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least two different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

35. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least three different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

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36. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least four different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

37. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least ten different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

38. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least twenty-five different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

39. (Withdrawn) The substrate of claim 33, wherein the substrate has immobilized thereon at least one hundred different nucleic acids each comprising a different nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-560 and complements thereof.

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